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Acid-base and copper-binding properties of three organic matter fractions isolated from a forest floor soil solution

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ABSTRACT.

Vast amounts of knowledge about the proton- and metal-binding properties of dissolved organic matter (DOM) in natural waters have been obtained in studies on isolated humic and fulvic (hydrophobic) acids. Although macromolecular hydrophilic acids normally make up about one-third of DOM, their proton- and metal-binding properties are poorly known. Here we investigated the acid-base and Cu-binding properties of the hydrophobic (fulvic) acid fraction and two hydrophilic fractions isolated from a soil solution. Proton titrations revealed a higher total charge for the hydrophilic acid fractions than for the hydrophobic acid fraction. The most hydrophilic fraction appeared to be dominated by weak acid sites, as evidenced by increased slope of the curve of surface charge versus pH at pH values above 6. The titration curves were poorly predicted by both Stockholm Humic Model (SHM) and NICA-Donnan model calculations using generic parameter values, but could be modelled accurately after optimisation of the proton-binding parameters ($\text{pH} \leq 9$). Cu-binding isotherms for the three fractions were determined at pH values of 4, 6 and 9. With the optimised proton-binding parameters, the SHM model predictions for Cu binding improved, whereas the NICA-Donnan predictions deteriorated. After optimisation of Cu-binding parameters, both models described the experimental data satisfactorily. Iron(III) and aluminium competed strongly with Cu for binding sites at both pH 4 and pH 6. The SHM model predicted this competition reasonably well, but the NICA-Donnan model underestimated the effects significantly at pH 6. Overall, the Cu-binding behaviour of the two hydrophilic acid fractions was very similar to that of the hydrophobic acid fraction, despite the differences observed in

proton-binding characteristics. These results show that for modelling purposes, it is essential to include the hydrophilic acid fraction in the pool of 'active' humic substances.

Keywords: copper; dissolved organic carbon, fractionation, isolation, hydrophobic acid, hydrophilic acid, transphilic acid, iron, aluminium, competition.

1. INTRODUCTION

Metals in natural waters generally exist in different physico-chemical forms (species). They can be sorbed onto suspended colloidal particles or bound by various dissolved ligands that are either organic or inorganic in nature. Knowledge of the speciation of a metal is essential in order to assess (i) the bioavailability or toxicity of the metal; and/or (ii) the mechanism(s) controlling its transport in the soil or aquatic system. The bioavailability differs markedly between different metal species. Many researchers have found evidence in support of the ‘free ion activity model’, where the toxicity or bioavailability is assumed to be related to the activity of the free, hydrated form of the metal (Allen *et al.*, 1980; Hue *et al.*, 1986). Regarding transport, the formation of soluble organic complexes has been shown to be an important mechanism for the mobilisation of many heavy metals in soils (Bergkvist *et al.*, 1989; Berggren, 1992b; Berggren, 1992a). Thus, any model describing the transport and bioavailability of metals in soils – and waters – has to consider the speciation in the aqueous phase.

For many metals, such as copper (Cu), aluminium (Al), iron (Fe), lead (Pb) and mercury (Hg), macromolecular organic acids constitute the major ligand in fresh water and soil solution (Tipping, 2002). The macromolecular acids are commonly divided into hydrophobic and hydrophilic acids based on how they react with an XAD-8 resin (Leenheer and Huffman Jr., 1976; Leenheer, 1981). Hydrophobic acids are defined as the fraction of dissolved organic matter that – following a uniform standard procedure – is retained by an XAD-8 resin column and desorbs on (back-)flushing the column with sodium hydroxide. For solutions, the compounds isolated in this way are also referred to as fulvic acids (Aiken *et al.*, 1985). The compounds that pass through the XAD-8 resin

column and are subsequently retained by a strongly basic anion exchanger are defined as hydrophilic acids. In soil solution and most other natural waters, the hydrophobic acids constitute about 50% and the hydrophilic acids about 30% of total dissolved organic carbon (Leenheer and Huffman Jr., 1976; Aiken *et al.*, 1992; Malcolm and MacCarthy, 1992).

The vast majority of research on metal binding has been performed on isolated humic and fulvic acids (Tipping, 1993b; Pinheiro *et al.*, 1999; Pinheiro *et al.*, 2000; Christl *et al.*, 2001; Milne *et al.*, 2003). In recent years, sophisticated models have been developed that are able to describe the acid-base chemistry and metal complexation properties of isolated humic and fulvic acids in a general way. The most frequently applied models include Model VI (Tipping, 1998), NICA-Donnan (Kinniburgh *et al.*, 1999) and SHM (Gustafsson, 2001). In the NICA-Donnan model, continuous sorption functions are used to describe proton and metal binding, whereas in Model VI and SHM binding occurs at discrete sites. Model VI and its precursor Model V (Tipping and Hurley, 1992) have been applied to a range of metals, including the alkaline earths, aluminium and transition metals (Tipping and Hurley, 1992; Tipping, 1993a; Tipping, 1993b; Tipping, 1998). It was found in applying these models to proton and metal binding data that there were distinct differences between fulvic and humic acids, but that there was no consistent dependence on the source of the two materials – soil, water, etc. (Tipping, 1998). Thus, it seems to be possible to define reasonable, consistent, general (average) binding characteristics for each of the two groups, something that is crucial for a wider application of models on natural waters.

Although hydrophilic acids constitute about one-third of the bulk of DOC in most natural waters, there are currently very few published studies on the metal-binding properties of these compounds. An early study showed that the binding strength of hydrophilic acids for chromium(III) and Cu exceeded that of hydrophobic acids by a factor of 2-8 (Guggenberger *et al.*, 1994). This was supported by structural characterisations made using ^{13}C NMR, which indicated that hydrophilic acids have a higher COOH (carboxylic) content than hydrophobic acids (Vance and David, 1991; Guggenberger *et al.*, 1994). However, in a more recent study by Croué *et al.* (2003) no major differences were observed in the Cu-binding properties of hydrophobic and hydrophilic acids. These conflicting results could be due to the fact that different experimental techniques were used in the two studies cited and/or that the sources of dissolved organic matter differed. Accordingly, more research is needed to investigate the metal-binding properties of hydrophobic and hydrophilic acids from different sources and for a greater number of metals. As pointed out by Dudal and Gérard (2004), improvement of the capacity of chemical equilibrium models to deal with non-humic substances, including hydrophilic acids, constitutes an important research direction.

In this work we studied three fractions of dissolved organic matter isolated from a forest floor soil solution sampled at the Asa experimental site, which has previously been well described and is currently being used for DOC leaching and metal-binding experiments (Fröberg *et al.*, 2003; Fröberg *et al.*, 2005; Gustafsson *et al.*, 2007). In addition to the most commonly studied fraction (fulvic acids/hydrophobic acids), we investigated two hydrophilic fractions from the same soil solution. Focus was on characterisation of acid-base and Cu-binding properties, as well as quantifying

competition effects of Fe(III) and Al on Cu binding. We also tested the ability of two geochemical models to describe the experimental data, both with and without parameter optimisation.

2. MATERIALS AND METHODS

Table 1. Chemical parameters of the soil solution sample taken from the Asa experimental site in southern Sweden

	Ca	Mg	Na	Al	Fe	Cd	Cu	Zn	Pb
							($\mu\text{g L}^{-1}$)		
Asa-1	44	35	345	134	95	<0.01	3.14	1.14	0.18
Asa-2	140	12	1291	361	274	<0.01	1.01	1.62	0.27

	Cl	NO₃-N	PO₄-P	SO₄-S	DOC
		(mg L^{-1})			
Asa-1	3.57	0.09	0.69	0.6	39.0
Asa-2	2.98	0	0.25	0.41	73.3

2.1. Sampling

The soil solution was collected from the O horizon in a Haplic Podsol (Typic Haplorthod - Soil Survey Staff, 2006) using zero-tension lysimeters. Samples were taken on two occasions (May 2005 and August 2005), filtered ($< 0.2 \mu\text{m}$, Supor 200) and stored in glass bottles at $+2^\circ\text{C}$ (Table 1). The sampling site was located in a Norway spruce forest at Asa Research Station in southern Sweden. Dissolved organic matter (DOM) in the O horizon solution from this site has previously been characterised in detail (Fröberg *et al.*, 2003).

Figure 1. Flowchart for fractionation and isolation of hydrophobic, transphilic and hydrophilic fractions of dissolved organic matter, DOM.

2.2. Isolation

A schematic overview of the entire isolation procedure is presented in Figure 1. An Amberlite® XAD-8/XAD-4 tandem was used during the isolations (Malcolm and MacCarthy, 1992). As there is no distinct boundary separating the different fractions, one has been operationally defined using a so-called distribution coefficient, k' :

$$V_E = V_0(1 + k')$$

where V_E is the volume of sample passing through the column, and V_0 is the void volume (i.e. porosity x bed volume). In other words, k' is a measure of the relationship between the sample volume passing through the column and the resin volume. We used a value of $k' = 100$ for both columns, similarly to Croué *et al.* (2003) and Guggenberger and Zech (1994).

Prior to isolation, the soil solution samples were cation-exchanged using an H^+ -saturated Bio-Rad AG-MP-50[®] column to remove trace metals and major cations from the solution. Solutions from both sampling occasions were then mixed and diluted to obtain a total volume of 5 L, containing 20 mg L⁻¹ DOC. By this procedure, the hydrophobic/hydrophilic split becomes independent of the initial DOC concentration (Qualls and Haines, 1991). Diluted samples were then pH-adjusted to 2 and passed through the columns containing XAD-8 and XAD-4, using a flow rate of 4 mL min⁻¹ (Malcolm and MacCarthy, 1992). Next, both columns were flushed with a 0.1 M NaOH solution and the column effluents were collected separately. The fraction that was isolated from the XAD-8 column was designated hydrophobic acids (HoA, Leenheer and Huffman Jr., 1976), while the fraction that was sorbed onto the XAD-4 column and successively desorbed by 0.1 M NaOH was designated transphilic acids (TiA, Croué *et al.*, 2003). The fraction which passed through both the XAD-8 and XAD-4 columns was designated hydrophilic compounds (HiC). To facilitate the isolation, the hydrophilic compounds were concentrated tenfold using rotor evaporation (Heidolph Laboroto 4000;

55 °C, -0.95 bar). The concentrate was then dialysed to remove excess salts and low molecular weight organic compounds (Spectrum Spectropor Biotech CE, MWCO 500). The isolated fractions were freeze-dried and stored in an exicator, in 250 mL polyethylene bottles covered with filter paper. A total of 12 isolations were made, yielding total amounts of 1100, 180 and 100 mg of HoA, TiA and HiC, respectively.

2.3. Chemical analysis

Cations and anions in solutions were analysed by ICP-AES (Jobin-Yvon JY24 ICP), ICP-MS (Perkin Elmer ELAN 6100 DRC) and ion chromatography (Dionex DX-120). Dissolved organic carbon was determined using a fully automated Shimadzu TOC-5000A analyser.

Elemental analyses of the isolated fractions were performed at Mikro Kemi AB in Sweden. Analyses of C, H, N and S (percentages) were based on the principle of combustion and had a precision of 0.3 (C), 0.17 (H and N) and 0.2 (S) %-units. Each sample was analysed in duplicate and the duplicates were not allowed to deviate by more than 0.10%-units for a content of 5.00-9.99%, 0.20 for a content of 10.0-24.9%, or 0.30 for a content of 25.0-90.0%. Pyrolysis was used for analysis of O, with a reported precision of 0.3 %-units and a maximum duplicate deviation of 0.4 %-units.

UV absorbance measurements were made on a Shimadzu UV-1201V spectrophotometer, using a 1 cm quartz cuvette at wavelengths of 254 and 260 nm. At those wavelengths natural organic matter absorbs strongly, giving increased sensitivity, and strongly correlates with the aromatic carbon content of organic matter (Dilling and Kaiser, 2002; Weishaar *et al.*, 2003). Measurements were made for each fraction, on solutions

containing 100 mg L⁻¹ DOM. Absorbance values were normalised for DOC content, yielding units of m⁻¹ L (mg C)⁻¹.

2.4. Proton and copper titrations

Proton titrations were performed using a TIM900 Titration Manager coupled with an ABU900 Autoburette (both Radiometer Copenhagen), using a Radiometer glass electrode (GK2401C) for pH measurements. A customised program was defined and tested against the results of some manual titrations for validation of the procedure and equipment used. The settings used for running the titration program were as follows: burette speed 0.5 mL min⁻¹, maximum dose per addition 0.250 mL, stirring delay 300 s, stability defined as $\Delta\text{pH} < 0.1 \text{ mpH s}^{-1}$, and maximum stability time 300 s. Acid-base titrations were performed on 25 mL sample solutions at $I = 0.1 \text{ M}$ and $I = 0.003 \text{ M}$ (NaNO₃), under continuous purging with high-grade N₂. The titrant was a 0.002 M NaOH solution, degassed and purged with N₂ for 15 minutes prior to titration. All titrations were made on solutions contained DOM concentrations of 100 mg L⁻¹. Test runs performed with solutions containing 50, 100 and 200 mg L⁻¹ DOM showed consistent charge density versus pH curves at pH ≤ 9. Data obtained at higher pH values were found to be less reliable, probably due to CO₂ entering the system contributing to the buffering.

Copper titrations were performed using a TIM960 Titration Manager (Radiometer Copenhagen) combined with the Titramaster 85 software (Radiometer-Analytical, 2007). The equipment allowed for simultaneous connection of a pH (PHG201, Radiometer Copenhagen) and a Cu(II) ion selective electrode (ISECU25, Radiometer Copenhagen) against one reference electrode (REF251, Radiometer Copenhagen), so that an automated method could be defined and used during the titrations. Titrations were performed at pH

4.0, pH 6.0 and pH 9.0 and $I = 0.03 \text{ M}$ (NaNO_3). A 30 mL aliquot of the sample was first adjusted to approximately the desired pH using a 0.1 M NaOH solution, and then fine-adjusted to the exact pH using a 0.01 M NaOH solution. The solutions were then subjected to titration with $\text{Cu}(\text{NO}_3)_2$, using three different titrant concentrations (0.1, 1.0 and 10.0 mM). This prevented the total volume of solution added from altering the DOC concentration and ionic strength excessively. Manual Cu additions were made using calibrated pipettes, followed by a three-step automated TIM960 program during which (i) pH was raised back to the desired value (within 0.01 units) using automated end-point titrations with 0.01 M NaOH and burette speeds of $0.01\text{-}0.1 \text{ mL min}^{-1}$; (ii) actual pH was measured; and (iii) Cu activity was measured. Stability criteria for the pH and Cu measurements were $\Delta\text{pH} < 0.1 \text{ mpH min}^{-1}$ and $\Delta\text{EMF} < 0.1 \text{ mV min}^{-1}$, respectively, with maximum stability time set at 300 s. Equilibrium under the criteria mentioned was found to occur within this time. The total concentration of Cu added ranged from $3 \times 10^{-7} \text{ M}$ to $5 \times 10^{-4} \text{ M}$. The exact pH values recorded were used for the modelling calculations. Solutions were continuously stirred and purged with a $\text{N}_2/\text{O}_2 (\text{g})$ mixture before and during the titration period to eliminate the interference of carbonate, and the temperature was kept constant at 25°C . The electrode potential measured with the Cu ion selective electrode (EMF_{Cu}) was converted to Cu^{2+} activity using a calibration curve obtained with a Cu-ethylenediamine buffer solution (Avdeef *et al.*, 1983). For the range of interest (pCu 4-14), the Cu^{2+} activities obtained were linearly related to calculations made in Visual MINTEQ (Gustafsson, 2007) with a slope of approx. 29 mV pCu^{-1} , which is in agreement with previously reported results (Benedetti *et al.*, 1995; Lu and Allen, 2002; Croué *et al.*, 2003). Input parameters for the model calculations were pH, total Cu and

ethylenediamine concentrations and concentrations of background electrolytes Na^+ and NO_3^- . Solution complexation constants used for the Visual MINTEQ calculations were taken from the NIST database (Smith *et al.*, 2007), but stability constants for Cu-ethylenediamine complexes were taken from Lomozik *et al.* (1997). The Cu-ethylenediamine calibrations yielded a very good linear response, as well as good agreement with the $\text{Cu}(\text{NO}_3)_2$ unbuffered calibration solutions.

Full calibrations were made at the start, in the middle and end of the experimental period and the electrode performance was checked against unbuffered $\text{Cu}(\text{NO}_3)_2$ calibration solutions (0.01, 0.001 and 0.0001 M) on a daily basis. No systematic drift occurred, but the results from the unbuffered samples indicated a small variation between measurements. The amount of Cu(II) bound to DOM was estimated as the difference between the total Cu and the sum of Cu^{2+} and Cu-inorganic complexes (as obtained from the measured Cu^{2+} activity and subsequent speciation modelling with Visual MINTEQ).

The standard deviation of $\log \{\text{Cu}^{2+}\}$ after the repeated measurements of unbuffered $\text{Cu}(\text{NO}_3)_2$ calibration solutions over time was found to be 0.05. Because this uncertainty affected the calculated amounts of Cu bound to DOM, particularly when the fraction of bound Cu was small, a Monte-Carlo simulation was performed in which 3 000 values of total Cu were generated at random to calculate values of bound Cu using Visual MINTEQ. This analysis showed that the standard deviation of $\log [\text{Cu bound}]$ was 0.05 at 50% bound Cu(II), and that it increased steeply with decreasing fraction of Cu bound. Hence, when modelling Cu-binding isotherms (see below), titration points at which calculated values of bound Cu(II) were < 50% of total Cu were deemed uncertain and excluded from data treatment.

Copper titrations with Fe and Al were carried out at pH 4 and 6. Visual MINTEQ was used for preliminary estimation of Al and Fe speciation, and the concentrations of Fe and Al at both pH values were chosen so that precipitation of Fe(III) and Al (hydr)oxides was avoided. The amounts of Fe and Al added were 0.9 mM for pH 4 and 0.09 mM for pH 6.

2.5. Calculations

In proton titrations, the surface charge of the organic material was derived from the titration data, using the following charge balance equation for each point of the titration curve

$$[\text{Org}^-] = [\text{H}^+] + [\text{Na}^+] - [\text{OH}^-] - [\text{NO}_3^-].$$

Copper titration data were used to calculate the total amount of dissolved inorganic Cu from the measured free Cu activity and pH. The amount of Cu bound was calculated from the difference between the total Cu concentration and the total dissolved inorganic Cu concentration.

2.6. Modelling

Experimental data were compared with model predictions using both the Stockholm Humic Model (Gustafsson, 2001) and the NICA-Donnan model (Kinniburgh *et al.*, 1996). Model calculations were performed using Visual MINTEQ (SHM) and ECOSAT (NICA-Donnan). Input parameters for the acid-base titrations were pH, DOC concentration (converted to DOM for NICA-Donnan) and total concentrations of the

background electrolytes Na^+ and NO_3^- . Input parameters for the Cu titrations were pH, Cu^{2+} activity, DOC concentration (converted to DOM for NICA-Donnan) and total concentrations of the background electrolytes. Initial studies made use of generic parameters for fulvic acid with both the SHM (Gustafsson, 2001) and NICA-Donnan (Milne *et al.*, 2001; Milne *et al.*, 2003) model. DOM/DOC ratios were calculated from the elemental analysis (Table 2).

Table 2. Elemental analyses of organic components in the hydrophobic acid (HoA), transphilic acid (TiA) and hydrophilic compound (HiC) fractions (element analyses on fractions corrected for inorganic contents)

Fraction	C	N	O	S	H	P	Inorganic residue ^{a)}	C/O	C/N	C/H
	% (w/w)						% (w/w)	mass ratio		
HoA	46.2	0.8	41.1	0.3	4.1	≤ 0.5	1.7	1.1	61.5	11.3
TiA	34.5	0.8	38.2	0.4	3.7	≤ 0.5	1.9	0.9	43.1	9.5
HiC	27.5	1.0	36.9	0.5	3.5	≤ 0.5	17.7	0.7	28.9	7.9

^{a)} Inorganic residue was determined by dissolving 100 mg L^{-1} DOM and calculating the sum of major cations and anions (Na, Al, Fe, Cl, SO_4 , PO_4).

Table 3. Results of chemical analyses of the hydrophobic acid (HoA), transphilic acid (TiA) and hydrophilic compound (HiC) fractions dissolved in deionised water (100 mg L^{-1}). SUVA=specific ultraviolet absorbance

	DOC	Fe	Al	SUVA - 260nm	SUVA - 254nm
	mg L^{-1}			$[\text{m}^{-1} \text{L (mg C)}^{-1}]$	
HoA	47.5	0.041	0.028	4.2	4.4
TiA	36.2	0.049	0.012	3.2	3.3
HiC	26.6	0.341	0.678	2.2	2.4

The models were then optimised to the experimental data, using root-mean-squared error (rmse) values to find optimal fits. Proton binding was optimised to the charge versus pH curves, and Cu-binding isotherms were used to optimise Cu-binding parameters. The apparent model charge was calculated as the difference between surface charge and the concentration of protons in the gel (SHM) or Donnan (NICA-Donnan) layer. For the HiC fraction, corrections for the complexation and hydrolysis of Al and Fe were found to be necessary because of the relatively high Al and Fe(III) concentrations of this fraction (Table 3). For example in the SHM model, the correction was made according to the following proton balance:

$$[\text{Charge}] = [\text{RO}^-] + 2 \cdot [(\text{RO})_2\text{Al}^+] + 2 \cdot [(\text{RO})_2\text{Fe}^+] + 3 \cdot [(\text{RO})_2\text{AlOH}] + 3 \cdot [(\text{RO})_2\text{FeOH}] + [\text{RO-AlOH}^{+2}\text{G}] + [\text{RO-FeOH}^{+2}\text{G}] - [\text{RO-H}^+\text{G}] + [\text{AlOH}^{+2}] + 2 \cdot [\text{Al}(\text{OH})_2^+] + 3 \cdot [\text{Al}(\text{OH})_3] + 4 \cdot [\text{Al}(\text{OH})_4^-] + [\text{FeOH}^{+2}] + 2 \cdot [\text{Fe}(\text{OH})_2^+] + 3 \cdot [\text{Fe}(\text{OH})_3] + 4 \cdot [\text{Fe}(\text{OH})_4^-]$$

where RO indicates a dissociated functional group, either carboxylic or phenolic, G indicates gel phase and the stoichiometry for each species corresponds to the molar concentration of H^+ released when forming this species. The calculated charge is thus an estimate of the total number of H^+ released during a base titration, which is only partly due to the acid dissociation of DOM. Therefore the optimised values for the number of DOM binding sites for the HiC fraction is slightly more uncertain than for the other fractions, since the former involves calculation from estimated concentrations of species that contain Al and Fe(III).

Optimisation of the SHM proton-binding parameters was carried out manually in a trial-and-error fashion in Visual MINTEQ, whereas ECOSAT was combined with the FIT package (Kinniburgh, 1993) for optimisation of the NICA-Donnan parameters.

Comment [JvS1]: Jag har tagit bort tabell 4, som beskriver alla parametrar – allt detta finns ju i tidigare publikationer. Av samma skäl har jag tagit bort två stycken nedan, som beskriver optimaliseringen i SHM och ND – trial-and-error talar för sig och proceduren för ND har tidigare beskrivits av Benedetti.

3. RESULTS AND DISCUSSION

3.1. Composition of isolated fractions

Table 4. Overview of average isolation results (n=12) and recovery percentages of the hydrophobic acid (HoA), transphilic acid (TiA) and hydrophilic compound (HiC) fractions measured prior to freeze-drying as DOC.

HoA	TiA	HiC ^{a)}	HiC ^{b)}	Total recovery ^{b)}
(% of total DOC)				
58.8 ± 4.0	9.3 ± 1.1	18.2 ± 1.6	13.8 ± 2.9	81.8

^{a)} recovery before dialysis

^{b)} recovery after dialysis (MWCO = 500)

An overview of isolation results is presented in Table 4. The hydrophobic acid fraction accounted for 59% of total DOC and was the dominant fraction, which was in good agreement with the value obtained by Leenheer and Huffman Jr. (1976, approximately 62% hydrophobic acids) and that by Martin-Mousset *et al.* (1997, 51-62% in reservoir waters). The transphilic acid fraction made up 9% of total DOC, which was lower than the 20% obtained by Malcolm and MacCarthy (1992, 20% in Norway lake water) and the 14-21% obtained by Martin-Mousset *et al.* (1997, 14-21% in reservoir waters). The hydrophilic fraction, which passed through both the XAD-8 and XAD-4 columns, constituted 18% of total DOC. Of this fraction, 24% was lost during dialysis (MWCO 500), corresponding to 4% of total DOC. Thus, the three fractions on which the metal and proton binding experiments were performed constituted 82% of total DOC.

Elemental analysis showed decreasing C/O, C/N and C/H ratios in the order HoA, TiA and HiC (Table 2). Inorganic residues were determined through wet chemical analysis

and the low contents in the hydrophobic and hydrophilic fractions indicate that the isolation procedure efficiently removed inorganic components from the sample. The hydrophilic fraction contained a significantly higher inorganic residue content as a result of the fact that (i) this fraction was not purified through sorption/desorption on a resin column; and (ii) the salt concentrations increased by approximately a factor of 10 during rotor evaporation. Although the rotor-evaporated sample was dialysed, not all salts were successfully removed during this step. The majority (approx. 62%) of the inorganic residue was present as sodium and chloride, the remainder being sulphate (23%), phosphate (11%), Al and Fe (both < 5%). The sum of the elemental masses and inorganic residues accounted for 94.6, 80.0 and 87.5% of HoA, TiA and HiC, respectively. The remaining mass was attributed to water content and analytical errors.

Fröberg *et al.* (2003) performed a full analytical fractionation according to Leenheer (1981) on soil solution collected from the same lysimeters as those used in the present study and found that the hydrophobic acids and neutrals constituted 58% and 5%, respectively, of total DOC and the hydrophilic acids, bases and neutrals constituted 30%, 3% and 4%, respectively, of total DOC. This distribution between fractions is well in line with that found for soil leachate collected below the O horizon in coniferous and hardwood forests in north-eastern USA and Europe (Vance and David, 1991; Guggenberger and Zech, 1994; Dai *et al.*, 1996). The value of 58% found by Fröberg *et al.* (2003) for the hydrophobic acid fraction is close to our figure of 59% (Table 4). In our preparative procedure, 14% of total DOC was irreversibly adsorbed by the XAD-8 and XAD-4 resins, i.e. could not be desorbed by 0.1 M NaOH. Since the hydrophobic neutrals comprise about 5% of total DOC at this site, it is likely that about 9% of total DOC was

irreversibly adsorbed by the XAD-4 resin. Thus, about 50% of DOC adsorbed by the XAD-4 resin could not be eluted by 0.1 M NaOH. A similarly low recovery was reported by Malcolm and MacCarthy (1992), who claimed that the whole fraction of DOC that was retained by the XAD-4 resin consisted of macromolecular hydrophilic acids. The irreversibly adsorbed part of these compounds probably constituted the most hydrophobic fraction of these acids. The strong interaction could arise from π - π bonding between the organic compounds and the aromatic matrix of XAD-4 (Malcolm and MacCarthy, 1992). The fraction passing through both the XAD-8 and XAD-4 resins and subsequently retained by the dialysis membrane probably consisted of the most hydrophilic fraction of 'hydrophilic acids' as classified by the Leenheer procedure. This would be in agreement with the fractionation data of Fröberg *et al.* (2003), suggesting that 30% of total DOC in soil solution at this site is 'hydrophilic acids'. Adding the TiA and the HiC fractions to the fraction irreversibly adsorbed by the XAD-4 resin (about 9%) produced a figure of 32%. It is likely that the fraction lost during dialysis mainly consisted of 'hydrophilic neutrals', i.e. mainly carbohydrates and polyfunctional alcohols (Guggenberger and Zech, 1994).

According to the principles of the isolation procedure, the hydrophobic character of the isolates should decrease in the order HoA > TiA > HiC, which is consistent with the trend of decreasing specific ultraviolet absorbance (SUVA) (Table 3). SUVA is a parameter that indicates the nature or quality of DOC in a given sample and has been used as a surrogate measurement for DOC aromaticity (Chin *et al.*, 1994; Weishaar *et al.*, 2003). The relative oxygen content (C/O ratio), on the other hand, increased in reverse order (HoA < TiA < HiC). Assuming that most oxygen is present in carboxylic (COOH)

and phenolic (OH) groups, this indicates an increased functional group density with decreasing hydrophobicity, as would be expected.

3.2. Acid-base properties and modelling

All three fractions showed a gradual increase in charge with increasing pH (Figure 2), which is in agreement with previously published results for humic and fulvic acids (Tipping, 2002). The ionic strength (electrostatic) effects were largest for the HoA fraction. The total charge of the HoA fraction was lower than that of the TiA and HiC fractions, which was in line with the observed C/O ratios. The slope of the proton titration curves for the HoA and TiA fractions was significantly steeper at pH values < 6 than at pH > 6, indicating that the number of carboxylic groups outnumbered the number of phenolic groups. The curve of the TiA fraction was slightly steeper in both sections, which suggests either a smaller width of distribution or a larger number of carboxylic and phenolic sites. The titration curve for the HiC fraction had a different character, as the slope actually increased at pH values > 6, suggesting that the fraction is dominated by phenolic-type binding sites.

Because of the high purity of the hydrophobic and transphilic fractions, the initial charge of the DOM fractions at the start of the titration experiments could be determined from charge balance. For the hydrophilic fraction, on the other hand, the presence of Al and Fe gave cause for caution. However, model simulations showed that the initial organic charge was not significantly influenced by Fe and Al at these concentrations. For the sake of simplicity we therefore assumed that the initial organic charge could be calculated from the charge balance, without explicitly accounting for Fe and Al for this fraction. The transphilic and hydrophilic acids were characterised by a higher initial

charge than the hydrophobic acids. The charge in the titrated pH interval increased in the order $\text{HiC} > \text{TiA} > \text{HoA}$, and would be even more prominent if expressed in mmol g^{-1} DOC rather than mmol g^{-1} DOM. Similarly, Dai *et al.* (1996) and Croué *et al.* (2003) reported a higher charge for the hydrophilic and transphilic acid fraction, respectively, in comparison to the hydrophobic acid fraction. Dai *et al.* (1996) reported that the hydrophilic acid fraction contained the highest proportion of C as carboxylic groups, which is in good agreement with the steeper curves for the TiA and HiC fractions at pH values < 6 in the present study.

Figure 2. Acid-base titration and SHM model curves for the hydrophobic acid (HoA), transphilic acid (TiA) and hydrophilic compound (HiC) fractions at $I = 0.1 \text{ M}$ and $I = 0.003 \text{ M}$. Red lines represent model predictions with generic parameters; green lines represent model predictions with optimised parameters. Lower right figure: modelled contribution of Fe and Al to total calculated charge, using optimised parameters.

Figure 3. Acid-base titration and NICA-Donnan model curves for the hydrophobic acid (HoA), transphilic acid (TiA) and hydrophilic compound (HiC) fractions at $I = 0.1$ M and $I = 0.003$ M. Red lines represent model predictions with generic parameters; green lines represent model predictions with optimised parameters. No fit was obtained for the HiC fraction.

The SHM (Figure 2) and NICA-Donnan (Figure 3) models produced comparable titration curves when using the generic parameter values, which is hardly surprising given the fact that both sets of parameters were derived from the same data. However, neither the SHM nor the NICA-Donnan model performed very well in describing the HoA titration results. The initial charge was predicted reasonably well, but the slope of the first part of the experimental curve was less steep than predicted. Consequently, the total charge at the endpoint of the titration was overestimated. In other words, the hydrophobic acid in this study had a lower content of acidic groups than the average fulvic acid.

Table 5. Acid-base and Cu(II) complexation constants in the SHM model for generic fulvic acid and for the hydrophobic acid, transphilic acid and hydrophilic compounds fractions

SHM				
	Generic fulvic acid	Hydrophobic acid	Transphilic acid	Hydrophilic compound
Acid-base parameter^{a)}				
n_{tot} (mol kg ⁻¹)	7.02	5.92	6.96	7.55
n_A (mol kg ⁻¹)	5.40	4.21	5.27	4.58
n_B (mol kg ⁻¹)	1.62	1.71	1.69	2.97
$\log K_A$	-3.51	-2.85	-2.9	-2.35
$\log K_B$	-8.81	-7.91	-7.4	-7.95
ΔpK_A	3.48	3.55	2.9	5.5
ΔpK_B	2.49	3.1	2.7	2.9
g_f	0.72	1.0	0.82	0.8
RMSE		0.0542	0.0570	0.1108
Cu complexation^{a)}				
$\log K_{Cu,m}$	-0.8	-0.81	-0.68	-0.6
$\log K_{Cu,b}$	-5.81	-5.89	-6.09	-5.96
$\log K_{CuOH,b}$	-13.4	-13.64	-13.71	-18.4
ΔLK_2	1.4	1.3	1.4	1.4
Fe(III)/Al complexation^{b)}				
$\log K_{Fe,b}$	-2.0			
$\log K_{FeOH,b}$	-5.8			
ΔLK_2	1.0			
$\log K_{Al,b}$	-4.2			
$\log K_{AlOH,b}$	-9.3			
ΔLK_2	1.0			

^{a)} from Gustafsson & van Schaik (2003); values were fixed during optimisation; ^{b)} Complexation constants for Fe(III) were constrained from the data sets of Liu and Millero (1999) and Tipping *et al.* (2002), and for Al the constants were optimised from the data sets treated by Milne *et al.* (2003).

However, the slope of the second part of the curve, represented by sites of type B and type 2 in the respective models, showed good agreement between model and experimental results. In order to improve the model results, the total number of binding

sites and the value of the binding constants of both the carboxylic and phenolic type sites needed to be lowered, indicating more strongly acid characteristics than assumed with the generic values (Table 5 & 6).

The fraction of type B binding sites was therefore increased from 23% to approx. 29%. For the SHM model, the distribution term, ΔpK , was increased for both types of sites, indicating larger site heterogeneity. Similar adjustments were required for the heterogeneity parameter in the NICA model, p , for sites of type A. For sites of type B, both the heterogeneity and the non-ideality parameter had to be fixed to obtain valid optimisation results. As discussed above, the ionic strength effect was larger than for the other fractions, which was reflected in the optimised values for g_f and b in the SHM and NICA-Donnan model, respectively.

An interesting finding was that the default model calculations gave rather good predictions for the TiA fraction, despite the fact that the generic parameter values were derived for data on fulvic (hydrophobic) acids. The SHM model gave a good fit after the total amount of binding sites was increased and the fraction of type B binding sites was adjusted to approx. 24%; i.e. lower than found for the HoA fraction, but comparable to the generic FA value of 23%. The proton dissociation constants and distribution term values for carboxylic type groups were comparable to those found for the HoA fraction, but the ionic strength effect was weaker. The steeper slope of the second part of the charge curve was reflected by a higher value for the proton dissociation constant, combined with a lower distribution term value, of the phenolic type groups. The fitting procedure for the NICA-Donnan model also resulted in a higher total amount of binding sites, and the fraction of type 2 sites increased to 27%.

Table 6. Acid-base and Cu(II) complexation constants in the NICA-Donnan model for generic fulvic acid and for the hydrophobic acid, transphilic acid and hydrophilic compounds fractions

NICA-Donnan				
	Generic fulvic acid	Hydrophobic acid	Transphilic acid	Hydrophilic compound ^{d)}
Acid-base parameters^{a)}				
$Q_{max,tot}$ (mol kg ⁻¹)	7.74	6.56	7.98	
$Q_{max1,H}$ (mol kg ⁻¹)	5.88	5.09	5.81	
$Q_{max2,H}$ (mol kg ⁻¹)	1.86	1.47	2.17	
$\log K_1$	2.34	1.77	1.79	
$\log K_2$	8.6	7.89	7.29	
$n_{H,1}$	0.66	0.8	0.72	-
$n_{H,2}$	0.76	0.76 ^{c)}	0.91	
b	0.57	0.3	0.49	
p_1	0.59	0.33	0.54	
p_2	0.70	0.7 ^{c)}	0.63	
RMSE		0.0526	0.0459	
Cu complexation^{b)}				
$\log K_{Cu,1}$	0.26	-1.69	-1.04	
$\log K_{Cu,2}$	8.26	5.41	6.31	
$n_{Cu,1}$	0.53	0.54	0.58	-
$n_{Cu,2}$	0.36	0.49	0.47	
Fe(III)/Al complexation^{b,c)}				
$\log K_{Fe,1}$	6.00			
$\log K_{Fe,2}$	36.00			
$n_{Fe,1}$	0.25			
$n_{Fe,2}$	0.19			
$\log K_{Al,1}$	-4.11			
$\log K_{Al,2}$	12.16			
$n_{Al,1}$	0.42			
$n_{Al,2}$	0.31			

^{a)} from (Milne *et al.*, 2001); ^{b)} from (Milne *et al.*, 2003); ^{c)} values fixed during optimisation; ^{d)} no fit found

As expected, neither model performed well for the HiC fraction when the generic parameter values were used. No acceptable fit could be obtained with the fitting

procedure for the NICA-Donnan model, either with a fully adjustable parameter set or by fixing certain parameters. The optimised SHM parameters indicated a nearly identical total amount of binding sites as for the TiA fraction, but the fraction of phenolic type binding sites had to be increased considerably, to almost 40%. The proton dissociation constant for sites of type A was further decreased in order to correctly account for the observed initial charge, while the distribution term had to be increased significantly. For our model calculations, we assumed that all measured Fe and Al were fully active. Figure 2 illustrates the effect of Al and Fe hydrolyses on the total charge as calculated by the model.

3.3. Copper titrations

Copper titrations spanned a free copper activity range of about 8 orders of magnitude in the experiments (from pCu 4 to 12) and showed a strong pH dependence for all fractions, which was in agreement with previous findings for fulvic acids from various sources and for bottom ash leachate (Benedetti *et al.*, 1995; Croué *et al.*, 2003; van Zomeren and Comans, 2004; Sarathy and Allen, 2005; Olsson *et al.*, 2007). Free Cu was lower than 1% for all fractions and all Cu additions at pH 9, and lower than 10% for the HoA and TiA fractions at pH 6, at total added Cu(II) concentrations up to approximately $1 \cdot 10^{-4}$ M. For the HiC fraction, free Cu exceeded 10% at about half this concentration. At pH 4, free Cu varied from close to none up to nearly 100% for all three fractions. Free Cu, in other words, increased with decreasing pH, which is typical for a system in which Cu(II) ions and protons compete for binding sites.

Figure 4. Experimental and modelled Cu titrations of the HoA, TiA and HiC fractions at pH 4, 6, and 9. Red lines represent model predictions with default proton- and Cu-binding parameters; green lines represent model predictions with optimised proton-binding and default Cu-binding parameters; black lines represent model predictions with fully optimised proton- and Cu-binding parameters. Reported *rmse* values are for the fully optimised model predictions.

Experimental solutions were undersaturated with respect to inorganic $\text{Cu}(\text{OH})_2$ ($\log K_S = 9.29$ at 25 °C), with the exception of the points in the highest Cu concentration

range at pH 9 (these points were removed from the data). As other inorganic speciation forms were negligible at the pH values studied, it was apparent that the majority of the total Cu was bound in organic complexes. The pH-stat Cu titrations produced Cu-binding isotherms that were nearly linear when plotted on a log-log scale (Figure 4). The slope of the curves was much smaller than one, indicating binding-site heterogeneity, i.e. strong Cu complexation to a small number of sites (Benedetti *et al.*, 1995). At pH 6, the HoA and TiA fractions showed signs of saturation of the binding sites, as the slope of the curves declined.

The Cu-binding isotherms were described reasonably well with the SHM model, using the optimised proton binding parameters (Figure 4). Copper binding in the SHM model is described by three reactions:

where RO represents a binding site of carboxylic (A) or phenolic (B) type. Optimised fits required relatively small adjustments to the Cu-binding parameters, with the exception of the binding constant for the bidentate CuOH complex for the HiC fraction (Table 5). In general, the predictions using optimised proton-binding parameters were better than the predictions using generic parameters. Thus, the weaker Cu-binding

properties found for our three fractions compared with the average fulvic acid might partly be explained by stronger acid-dissociating groups.

The NICA-Donnan model accommodates monodentate and bidentate complex binding, described by two reactions:

Comment [JvS2]: Kanske tydliggöra hur stoichiometrin påverkas av n_H/n_i ?

where RO_1 and RO_2 represent binding sites of carboxylic (A) and phenolic (B) types, respectively. The average stoichiometry can be derived from the ratio of n_H/n_i .

In contrast to the SHM model, the NICA-Donnan predictions deteriorated considerably when the optimised proton-binding parameters were applied, overestimating Cu binding by about one order of magnitude at all pH values. Good fits were obtained by lowering the Cu-binding constants for both site types, thus decreasing Cu complexation and competition. The non-ideality of Cu-specific binding to sites of type 2 was found to be larger than assumed by the generic parameters, as indicated by a lower value for the non-ideality parameter n . Non-ideality can be due to various physicochemical interactions, e.g. metal-metal interactions between different sites or changes/differences in site accessibility (Benedetti *et al.*, 1996).

Direct comparison of the Cu-binding isotherms of the three fractions revealed that the slopes were nearly identical at all pH values (Figure 5, left), indicating the same kind of heterogeneity for all three fractions, involving similar functional groups.

Figure 5. Comparison of Cu-binding isotherms of the HoA, TiA and HiC fractions at pH 4, 6 and 9 (left); and effect of Fe and Al on the Cu-binding isotherm (right). Open and closed symbols represent duplicate titration data. Lines represent the optimised model fit for a system including or excluding Fe and Al.

However, the HiC fraction showed markedly weaker Cu binding than the other two fractions, particularly at pH 6 and pH 4. In an attempt to explain this observation, we simulated the Cu-binding isotherm for the HiC fraction, using the optimised parameters, in the presence and absence of Fe and Al (Figure 5, right). The simulation showed that the Cu-binding isotherm for the HiC fraction closely resembled those for the HoA and TiA fractions, in the absence of the competing Fe and Al ions. These observations are in good agreement with findings by Croué *et al.* (2003), who studied Cu binding by hydrophobic and transphilic acid fractions isolated from a South Platte River sample, and Olsson *et al.* (2007), who compared Cu binding in a cation-exchanged bottom ash leachate to Cu binding in the same sample after passage through an XAD-8 column. The eluate thus contained all but the hydrophobic fraction of the total DOM, and the results revealed that removal of the hydrophobic fraction did not alter the Cu-binding properties significantly. Guggenberger *et al.* (1994), on the other hand, reported that Cu binding by

hydrophilic acids 2-8-fold exceeded that by hydrophobic acids. However, those results were not obtained by studying Cu binding to isolated fractions, but by a speciation calculation based on measurements before and after passage through XAD-8 and cation exchange resins, i.e. by a kinetic discrimination between forms of the metal. Thus, possible artifacts due to alteration of original equilibria could have obscured the results obtained in that study.

Figure 6. Copper titrations of the HoA, TiA and HiC fractions at pH 4, 6 and 9 and in the absence and presence of Fe(III) as the competing ion. Triangles represent pH 6 data, diamonds represent pH 4 data; dashed lines show optimised model calculations in the

absence of Fe(III), solid lines show model predictions with Fe(III) competition. Left: SHM model. Right: NICA-Donnan model.

Figure 7. Copper titrations of the HoA, TiA and HiC fractions at pH 4, 6 and 9 and in the absence and presence of Al as the competing ion. Triangles represent pH 6 data, diamonds represent pH 4 data; dashed lines show optimised model calculations in the absence of Al, solid lines show model predictions with Al competition. Left: SHM model. Right: NICA-Donnan model.

3.4. Iron(III) and aluminium competition

Addition of Fe(III) or Al to the samples prior to titration resulted in a lowered degree of complexation (Figures 6 and 7), illustrating a significant competition effect of both Fe(III) and Al for all three fractions. Competition was most pronounced at pH 4, but it must be emphasised that the amounts of Fe(III) and Al added at pH 4 were ten times higher than the amounts added at pH 6. Model predictions of the Fe(III) and Al competition experiments were made using both models (Figures 6 and 7). Previously derived optimised proton- and Cu-binding parameters were used, whereas the Fe(III) and Al complex-binding constants were generic ones (Table 5). The SHM model slightly underestimated the competition effect of Fe at pH 4, but for Al the model predictions were very good. The NICA-Donnan model underestimated the competition effect for both Fe and Al at pH 4. However at pH 6, both models performed worse and showed most discrepancies, with the SHM model tending to overestimate both Fe and Al competition in most simulations, whereas the competition effect was severely underestimated using the NICA-Donnan model. We also tested the NICA-Donnan model using the Fe-binding parameters as suggested by Hiemstra & van Riemsdijk (2006) in a study on Fe(III) speciation in ocean water. This resulted in slightly improved model prediction, but Fe(III) competition was still underestimated in comparison to the experimental data (not shown).

The differences in model behaviour are not easily explained by a single factor, but rather may be due to a number of interacting factors caused by fundamental differences in the assumptions concerning metal-binding mechanisms and site heterogeneity in the two

models. In any case, the experimental data clearly indicated that both Fe(III) and Al compete for binding sites with Cu, which is in line with previous studies (Tipping *et al.*, 2002; Weng *et al.*, 2002; Tipping, 2005; Weber *et al.*, 2006). This has important implications for model simulations of field data – unless the simulation of competition effects by Fe(III) and Al are considered, model predictions will underestimate free Cu(II) ion concentrations, particularly at low total Cu values.

3.5. Geochemical implications

Often, the available data for natural waters are limited to values for pH, organic carbon content (DOC) and total concentrations of major cations and anions and trace metals. Therefore, the application of geochemical speciation models, such as Model VI, SHM or NICA-Donnan, requires an assumption to be made with respect to the ‘active’ concentration of organic matter (Tipping, 2002). Since carbon makes up on average about 50% by weight of humic matter, the concentration of active humic matter will be less than or equal to twice the DOC concentration. If experimental data are available, a common procedure is to fix all the model parameters at their default (generic) values, then attempt to fit the data by adjusting the concentrations of ‘active’ humic substances. Good agreement between observed and predicted free Cu(II) ion concentrations was achieved by Cabaniss and Shuman (1988) after adjustment of the ‘active’ concentration to about 50% of the actual DOM. Dwane and Tipping (1998) obtained best results with ‘active’ concentrations of 59 and 65% for an upland pool and lowland river, respectively, which was comparable to the optimal value of 69% found by Vulkan *et al.* (2000) and the 60% found by Holm *et al.* (1995). Although this approach seems to result in reasonably

consistent results between different studies, it would be more satisfactory to have analytical methods that yield the active organic content concentrations more directly (Tipping, 2002).

In this study, we showed that despite differences in proton-binding characteristics, the transphilic and hydrophilic fractions had very similar Cu-binding properties to the hydrophobic fraction. The observed differences were small, even more so in light of the variation observed in properties of fulvic acids isolated from different environments. Thus, while inclusion of the transphilic and hydrophilic fractions in the model calculations appears to be critical, it may not be necessary to distinguish between the fractions. Instead, the sum of the hydrophobic and hydrophilic acids (according to the Leenheer fractionation) could be used as a fixed model input, rather than optimising the amount of ‘active’ humic substance. As discussed above, it is likely that our two hydrophilic fractions (TiA, HiC) together make up the major fraction of the hydrophilic acids, defined according to Leenheer (1981). However, this study only contained data for one metal and one soil solution sample, and more research and data are essential to further test and validate this possibility.

4. CONCLUSIONS

- Using an XAD-8/XAD-4 tandem, in combination with dialysis, we successfully isolated three organic matter fractions from a forest floor soil solution, with a total recovery of 82%. The fraction isolated from the XAD-8 column is equivalent with the fulvic acid fraction, whereas the two hydrophilic fractions probably constitute the most hydrophilic part of the ‘hydrophilic acids’ obtained in a Leenheer fractionation scheme.
- The three fractions isolated were characterised by distinct, yet somewhat differing, proton-binding characteristics, as indicated by surface charge versus pH curves. Binding site heterogeneity and polyelectrolytic properties were observed for all three fractions. Despite the observed differences in proton-binding properties, the Cu-binding isotherms were very similar for all three fractions. Also, Fe(III) and Al had a similar competition effect on Cu binding in all three samples.
- In general, both the SHM and NICA-Donnan models could be adjusted to obtain good fits for data sets on both proton- and Cu- binding. Attempts to model the competition effects of Fe(III) and Al on Cu binding were more successful with the SHM model than the NICA-Donnan model.
- We propose that the Leenheer fractionation procedure could be used for an experimental determination of the ‘active’ humic substance content of a sample, defined as the sum of the hydrophobic and hydrophilic acid fractions. This would be preferable to an estimated value obtained from model optimisation. However,

more research on other types of waters and metals are needed to test this approach.

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REFERENCES

- Aiken, G. R., McKnight, D. M., Thorn, K. A., and Thurman, E. M., 1992. Isolation of hydrophilic organic-acids from water using nonionic macroporous resins. *Organic Geochemistry* **18**, 567-573.
- Aiken, G. R., McKnight, D. M., Wershaw, R. L., and MacCarthy, P., 1985. *Humic substances in soil, sediment and water: Geochemistry, isolation and characterization*. Wiley, New York.
- Allen, H. E., Hall, R. H., and Brisbin, T. D., 1980. Metal speciation. Effects on aquatic toxicity. *Environ. Sci. Technol.* **14**, 441-443.
- Avdeef, A., Zabronsky, J., and Stuting, H. H., 1983. Calibration of copper ion selective electrode response to pCu 19. *Anal. Chem.* **55**, 298-304.
- Benedetti, M. F., Milne, C. J., Kinniburgh, D. G., Riemsdijk, W. H. v., and Koopal, L. K., 1995. Metal ion binding to humic substances: Application of the non ideal competitive adsorption model. *Environ. Sci. Technol.* **29**, 446-457.
- Benedetti, M. F., Van Riemsdijk, W. H., Koopal, L. K., Kinniburgh, D. G., Gooddy, D. C., and Milne, C. J., 1996. Metal ion binding by natural organic matter: From the model to the field. *Geochim. Cosmochim. Acta* **60**, 2503-2513.
- Berggren, D., 1992a. Speciation of aluminum and cadmium in soil solutions from Podzols and Cambisols of S Sweden. *Water Air and Soil Pollution* **62**, 125-156.

- Berggren, D., 1992b. Speciation of copper in soil solutions from Podzols and Cambisols of S Sweden. *Water Air and Soil Pollution* **62**, 111-123.
- Bergkvist, B., Folkesson, L., and Berggren, D., 1989. Fluxes of Cu, Zn, Pb, Cd, Cr and Ni in temperate forest ecosystems - a literature review. *Water Air and Soil Pollution* **47**, 217-286.
- Cabaniss, S. E. and Shuman, M. S., 1988. Copper-binding by dissolved organic matter; II. Variation in type and source of organic matter. *Geochim. Cosmochim. Acta* **52**, 195-200.
- Chin, Y.-P., Aiken, G., and O'Loughlin, E., 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environ. Sci. Technol.* **28**, 1853-1858.
- Christl, I., Milne, C. J., Kinniburgh, D. G., and Kretzschmar, R., 2001. Relating ion binding by fulvic and humic acids to chemical composition and molecular size. 2. Metal binding. *Environ. Sci. Technol.* **35**, 2512-2517.
- Croué, J. P., Benedetti, M. F., Violleau, D., and Leenheer, J. A., 2003. Characterization and copper binding of humic and nonhumic organic matter isolated from the South Platte river: Evidence for the presence of nitrogenous binding site. *Environ. Sci. Technol.* **37**, 328-336.
- Dai, K. O., David, M., and Vance, G., 1996. Characterization of solid and dissolved carbon in a spruce-fir Spodosol. *Biogeochemistry* **35**, 339-365.
- Dilling, J. and Kaiser, K., 2002. Estimation of the hydrophobic fraction of dissolved organic matter in water samples using UV photometry. *Water Res.* **36**, 5037-5044.
- Dudal, Y. and Gérard, F., 2004. Accounting for natural organic matter in aqueous chemical equilibrium models: A review of the theories and applications. *Earth-Science Reviews* **66**, 199-216.
- Dwane, G. C. and Tipping, E., 1998. Testing a humic speciation model by titration of copper-amended natural waters. *Environment International* **24**, 609-616.
- Fröberg, M., Berggren, D., Bergkvist, B., Bryant, C., and Knicker, H., 2003. Contributions of Oi, Oe and Oa horizons to dissolved organic matter in forest floor leachates. *Geoderma* **113**, 311-322.
- Fröberg, M., Kleja, D. B., Bergkvist, B., Tipping, E., and Mulder, J., 2005. Dissolved organic carbon leaching from a coniferous forest floor -- a field manipulation experiment. *Biogeochemistry* **75**, 271-287.

- Guggenberger, G., Glaser, B., and Zech, W., 1994. Heavy-metal binding by hydrophobic and hydrophilic dissolved organic-carbon fractions in a Spodosol-a and Spodosol-b-horizon. *Water Air and Soil Pollution* **72**, 111-127.
- Guggenberger, G. and Zech, W., 1994. Dissolved organic carbon in forest floor leachates: Simple degradation products or humic substances? *The Science of The Total Environment* **152**, 37-47.
- Gustafsson, J. P., 2001. Modeling the acid-base properties and metal complexation of humic substances with the Stockholm Humic Model. *J. Colloid Interface Sci.* **244**, 102-112.
- Gustafsson, J. P., 2007, Visual MINTEQ ver 2.53, <http://www.lwr.kth.se/English/OurSoftware/vminteq/>.
- Gustafsson, J. P., Persson, I., Kleja, D. B., and van Schaik, J. W. J., 2007. Binding of iron(III) to organic soils: EXAFS spectroscopy and chemical equilibrium modeling. *Environ. Sci. Technol.* **41**, 1232-1237.
- Gustafsson, J. P. and van Schaik, J. W. J., 2003. Cation binding in a mor layer: Batch experiments and modelling. *Eur. J. Soil Sci.* **54**, 295-310.
- Hiemstra, T. and van Riemsdijk, W. H., 2006. Biogeochemical speciation of Fe in ocean water. *Marine Chemistry* **102**, 181-197.
- Holm, P. E., Christensen, T. H., Tjell, J. C., and McGrath, S. P., 1995. Speciation of cadmium and zinc with application to soil solutions. *J Environ Qual* **24**, 183-190.
- Hue, N. V., Craddock, G. R., and Adams, F., 1986. Effect of organic-acids on aluminum toxicity in subsoils. *Soil Science Society of America Journal* **50**, 28-34.
- Kinniburgh, D. G., Milne, C. J., Benedetti, M. F., Pinheiro, J. P., Filius, J., Koopal, L. K., and Riemsdijk, W. H. v., 1996. Metal ion binding by humic acid: Application of the NICA-donnan model. *Environ. Sci. Technol.* **30**, 1687-1698.
- Kinniburgh, D. G., Riemsdijk, W. H. v., Koopal, L. K., Borkovec, M., Benedetti, M. F., and Avena, M. J., 1999. Ion binding to natural organic matter: Competition, heterogeneity, stoichiometry and thermodynamic consistency. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **151**, 147-166.
- Kinniburgh, D. G., 1993. Technical report WD/93/23: FIT user guide. British Geological Survey.

- Leenheer, J. A., 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environ. Sci. Technol.* **15**, 578-587.
- Leenheer, J. A. and Huffman Jr., E. W. D., 1976. Classification of organic solutes in water by using macroreticular resins. *Journal of Research of the US Geological Survey* **4**, 737-751.
- Liu, X. and Millero, F. J., 1999. The solubility of iron hydroxide in sodium chloride solutions. *Geochim. Cosmochim. Acta* **63**, 3487-3497.
- Lomozik, L., Gasowska, A., and Bolewski, L., 1997. Noncovalent interactions in polyamine/nucleoside (or diamino-carboxylate) systems studied by potentiometric and NMR techniques. *Journal of the Chemical Society-Perkin Transactions 2*, 1161-1165.
- Lu, Y. F. and Allen, H. E., 2002. Characterization of copper complexation with natural dissolved organic matter (DOM) - link to acidic moieties of DOM and competition by Ca and Mg. *Water Res.* **36**, 5083-5101.
- Malcolm, R. L. and MacCarthy, P., 1992. Quantitative evaluation of XAD-8 and XAD-4 resins used in tandem for removing organic solutes from water. *Environment International* **18**, 597-607.
- Martin-Mousset, B., Croue, J. P., Lefebvre, E., and Legube, B., 1997. Distribution and characterization of dissolved organic matter of surface waters. *Water Res.* **31**, 541-553.
- Milne, C. J., Kinniburgh, D. G., and Tipping, E., 2001. Generic NICA-donnan model parameters for proton binding by humic substances. *Environ. Sci. Technol.* **35**, 2049-2059.
- Milne, C. J., Kinniburgh, D. G., van Riemsdijk, W. H., and Tipping, E., 2003. Generic NICA-donnan model parameters for metal-ion binding by humic substances. *Environ. Sci. Technol.* **37**, 958-971.
- Olsson, S., van Schaik, J. W. J., Gustafsson, J. P., Kleja, D. B., and van Hees, P. A. W., 2007. Copper(II) binding to dissolved organic matter fractions in municipal solid waste incinerator bottom ash leachate. *Environ. Sci. Technol.* **41**, 4286-4291.
- Pinheiro, J. P., Mota, A. M., and Benedetti, M. F., 1999. Lead and calcium binding to fulvic acids: Salt effect and competition. *Environmental Science and Technology* **33**, 3398-3404.
- Pinheiro, J. P., Mota, A. M., and Benedetti, M. F., 2000. Effect of aluminum competition on lead and cadmium binding to humic acids at variable ionic strength. *Environmental Science and Technology* **34**, 5137-5143.

- Qualls, R. G. and Haines, B. L., 1991. Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. *Soil Sci Soc Am J* **55**, 1112-1123.
- Radiometer-Analytical, 2007, Titramaster 85 titration software, http://www.radiometer-analytical.com/en_titralab_software.asp.
- Sarathy, V. and Allen, H. E., 2005. Copper complexation by dissolved organic matter from surface water and wastewater effluent. *Ecotoxicology and Environmental Safety* **61**, 337-344.
- Smith, R. M., Martell, A. E., and Motekaitis, R. J., 2007. *Nist standard reference database 46*. National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD.
- Soil Survey Staff, 2006, Keys to soil taxonomy, 10th ed., USDA-Natural Resources Conservation Service, Washington, DC
- Tipping, E., 1993a. Modeling ion-binding by humic acids. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*.
- Tipping, E., 1993b. Modeling the competition between alkaline earth cations and trace metal species for binding by humic substances. *Environ. Sci. Technol.* **27**, 520-529.
- Tipping, E., 1998. Humic ion-binding Model VI: An improved description of the interactions of protons and metal ions with humic substances. *Aquatic Geochemistry* **4**, 3-47.
- Tipping, E., 2002. *Cation binding by humic substances*. Cambridge University Press, Cambridge.
- Tipping, E., 2005. Modelling Al competition for heavy metal binding by dissolved organic matter in soil and surface waters of acid and neutral pH. *Geoderma* **127**, 293-304.
- Tipping, E. and Hurley, M. A., 1992. A unifying model of cation binding by humic substances. *Geochim. Cosmochim. Acta* **56**, 3627-3641.
- Tipping, E., Rey-Castro, C., Bryan, S. E., and Hamilton-Taylor, J., 2002. Al(III) and Fe(III) binding by humic substances in freshwaters, and implications for trace metal speciation. *Geochim. Cosmochim. Acta* **66**, 3211-3224.
- van Zomeren, A. and Comans, R. N. J., 2004. Contribution of natural organic matter to copper leaching from municipal solid waste incinerator bottom ash. *Environ. Sci. Technol.* **38**, 3927-3932.

- Vance, G. F. and David, M. B., 1991. Chemical characteristics and acidity of soluble organic substances from a northern hardwood forest floor, central Maine, USA. *Geochim. Cosmochim. Acta* **55**, 3611-3625.
- Weber, T., Allard, T., Tipping, E., and Benedetti, M. F., 2006. Modeling iron binding to organic matter. *Environ. Sci. Technol.* **40**, 7488-7493.
- Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., and Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* **37**, 4702-4708.
- Weng, L., Temminghoff, E. J. M., Lofts, S., Tipping, E., and Van Riemsdijk, W. H., 2002. Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil. *Environmental Science and Technology* **36**, 4804-4810.
- Vulkan, R., Zhao, F. J., Barbosa-Jefferson, V., Preston, S., Paton, G. I., Tipping, E., and McGrath, S. P., 2000. Copper speciation and impacts on bacterial biosensors in the pore water of copper-contaminated soils. *Environ. Sci. Technol.* **34**, 5115-5121.